FDA's Perspective on Qualification and Stability of Potency Standards for Therapeutic Protein Products

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Overview

- Definitions
- Qualification of potency standards
- Potency assignment
- How to establish “shelf-life” of potency standard
- The strategies for minimize potency drift for a new standard
- A case study: development of the first international standard for VWF concentrates

Definitions of Potency

US 21 CFR 600.3(s)

"The word potency is interpreted to mean the specific ability or capacity of the product, as indicated by appropriate laboratory tests or by adequately controlled clinical data obtained through the administration of the product in the manner intended, to effect a given result."

ICH Q6B

- Potency is the quantitative measure of biological activity based on the attribute of the product that is linked to the relevant biological properties
- Biological activity is defined as the specific ability or capacity of the product to achieve a defined biological effect

Definitions of Tests for Potency

US 21 CFR 610.10

"Tests for potency shall consist of either *in vitro* or *in vivo*, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in part 600.3(s) of this chapter."

ICH Q6B

- Reflect the *in vivo* mechanism(s) of action of the protein
- Bioassays
  - Animal based
  - Cell culture based
  - Biochemical based
- Other procedures, such as ligand/receptor binding assays may be acceptable

Why Do We Need Potency Standards?

- Facilitate understanding and interpretation of test results among different laboratories
- Improve and assure quality of product, e.g., reduce assay variations
- Allow comparison of test results from different methodologies (not always)
- Establish consensus amongst laboratories - intra- and inter-institutional consistency

Qualification of Potency Standards

- Characterization of Candidates
  - Identity, purity and formulation
    - Lots representative of production and clinical materials (ICH Q6B)
  - Assay performance
    - Potency against international or national standards, and comparison with test substances likely to be assayed
- Stability
- Acceptance Criteria
  - The integrity of the protein structure (represent the degree of purity of the test samples)
  - Linearity of dose-response over a wide range of concentrations
  - Minimal inter-assay, intra-assay, and inter-laboratory variability
  - Similar results between different assay methodologies
  - Stability over stressed and real time storage

Potency Assignment

- International/National Potency Standards
  - International/national collaborative study
    - Two or more candidates prepared by different methods
    - Each lab is asked to use its own assay methods together with a standardized method if available
    - Comparison with existing unit if available
- In-house (Primary or Working) Potency Standards
  - Institutional or global collaborative study
    - Two or more candidate lots
    - Calibrate against both international/national and current in-house potency standards
    - Comparison between SOP and methods used in clinical labs

Potency Assignment (Cont.)

- Standard Statistical Methods (the results be analyzed by a central lab)
  - A mean potency estimate for each lab
  - An overall weighted mean
    - Include results from all assays (except those which are statistically invalid)
- Quality Control
  - Institutional quality control unit on standards
  - International expert groups on standards, e.g., WHO Expert Committee on Biological Standardization

How to Establish “shelf-life” of Potency Standard?

- Accelerated Stability Study
  - -20 °C (storage condition), +4 °C, +20 °C, +37 °C, and +45 °C
    - Degradation mechanism
    - Extrapolations (ICH Q1E)
- SOP for Evaluating the Proposed “shelf-life”
  - e.g., periodic recalibration study

The Strategies for Minimize Potency Drift for a New Standard

- Reduce the Frequency of Preparing a New Standard
  - Stable primary potency standard
    - Formulation
    - Container closure, e.g., Ampoules vs. vials
    - Storage conditions
      - Freeze-dried -- moisture levels as low as acceptable for integrity and eliminate oxygen
      - Stored at –20 °C in the dark
- Minimize potency drift when implementing new generation of international/national standard
  - Know the developmental process of the new standard
    - Be part of the collaborative study
    - literatures
  - Consider global recalibration study to minimize institutional assay bias

Development of the 1st International Standard for VWF Concentrates

- Phase I: Qualification Studies
- Phase II: Production and Calibration of Standard

Proposed Use of Standard:

- VWF:Ag
- VWF:RCof
- VWF:CB

Phase I: Qualification Studies

- Five products from 5 different manufacturers had been used for the Phase I study.
- Three organizations (FDA, NIBSC and SSC) were involved with assessment of potency, purity, integrity, stability and assay performance of various VWF concentrates.
- Two candidates were selected for further development.

Phase I: Qualification Studies

Criteria for Selecting the Two Candidates

- Primary Criteria
  - Stability of the samples
    - Accelerated stability studies using functional and antigen measurements
    - Stability of VWF multimers
  - Parallelism of dose-response curves relative to all testing vWF concentrates

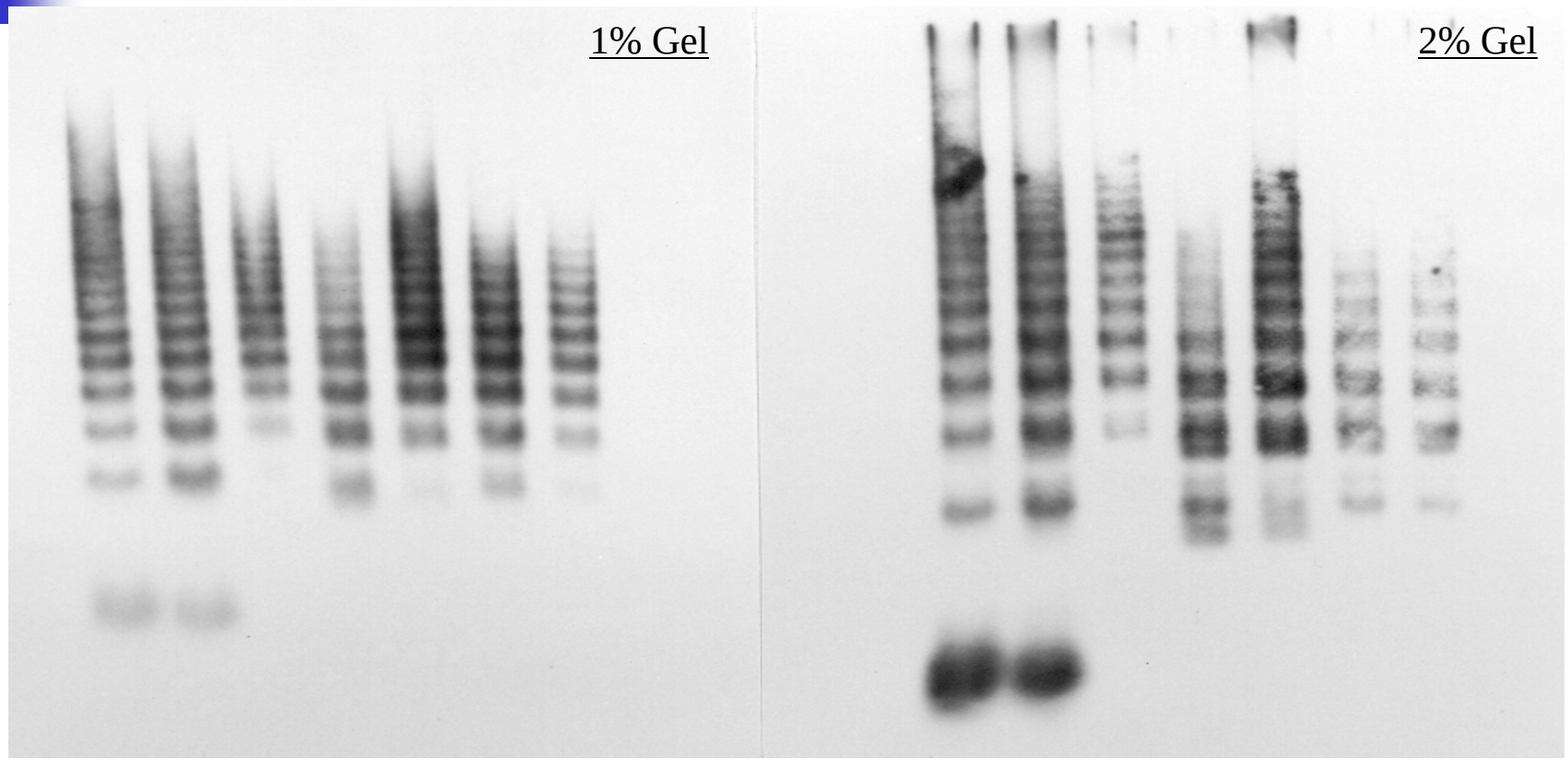
Phase I: Qualification Studies (Cont.)
Criteria for Selecting the Two Candidates

- Secondary Criteria
  - Similar results with different potency assay methodologies
  - The ratio between activity and antigen, e.g. close to one
  - The integrity of VWF multimer forms

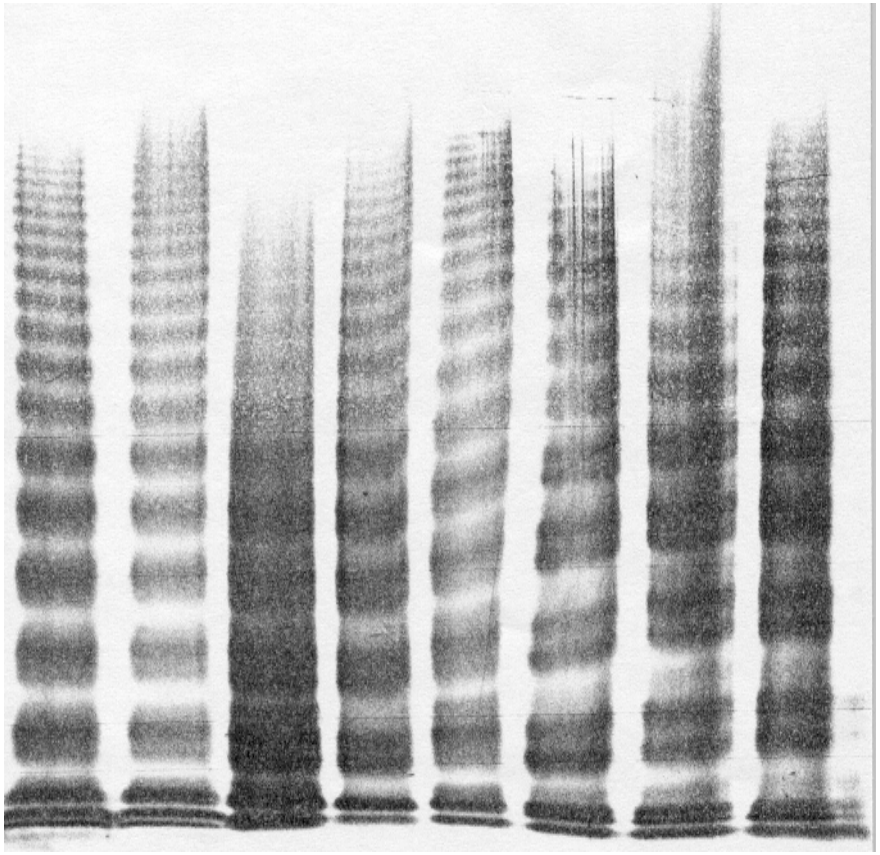
VWF Activities from 5 Different VWF Concentrates

Samples	vWF:Ag (IU/vial)	vWF:RCof (IU/vial)	vWF:CB (IU/vial)	RCof/Ag (%)	CB/Ag (%)	RCof/CB (%)
C-1	10.43	10.03	10.23	96	98	98
C-2	21.54	9.27	11.71	43	54	79
C-3	10.92	8.19	8.45	75	77	97
C-4	18.29	13.5	13.66	74	75	99
C-5	13.4	8.63	9.74	64	73	89

Variation of VWF Multimers Among Different Concentrates



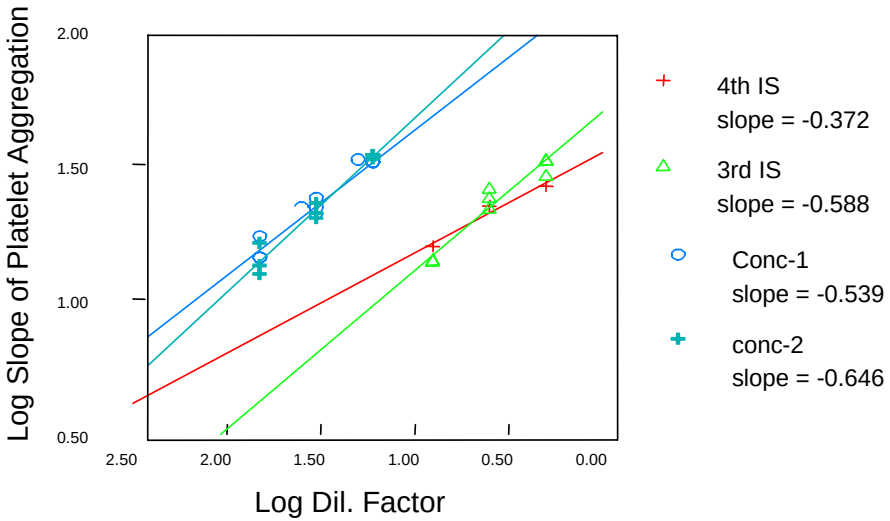
VWF Multimers
[% of the 4th IS (97/586)]

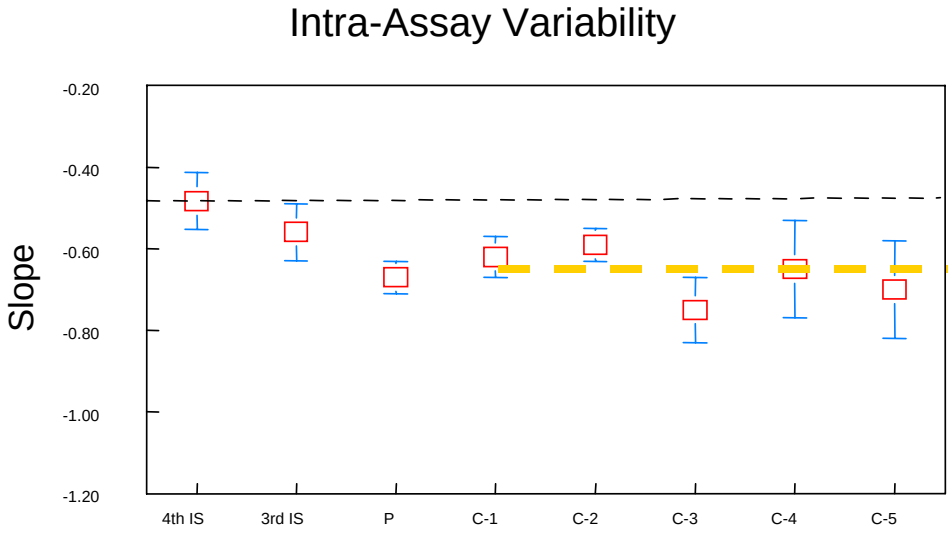


P IS C-2 NR C-1 C-5 C-4 C-3

Samples	≥15	≥10	≥5
C-1	36	62	87
C-2	NQ	49	74
C-3	40	50	84
C-4	NQ	38	69
C-5	25	52	82.5

Dose-Response Relationship
VWF:RCof Assays





Dose-Response Relationship

Samples	SLOPE					
	% mean (Rcof)	% mean (CB)		% mean (Ag)		
	FDA	LFB	NIBSC	FDA	LFB	NIBSC
C-1	94	110	100	99	99	104
C-2	89	99	104	82	100	102
C-3	112	92	97	116	100	101
C-4	99	104	102	99	97	99
C-5	106	95	96	105	103	94

Conclusions

- The slopes of the dose-response curves were not statistically different among C-1, C-3, C-4, and C-5.
- All 5 concentrates appear suitably stable for use as International Standards.

Therefore, the primary criteria, parallelism of the dose-response curves and stability, have very limited utility in the selecting process.

Selecting the Two Candidates

by the secondary criteria

		Specific Activity		Similarity	Multimers
Score*		RCof / Ag	CB / Ag	RCof / CB	% 4th IS
C-1	18	5	5	4	4
C-2	5	1	1	1	2
C-3	16	4	4	3	5
C-4	12	3	3	5	1
C-5	9	2	2	2	3

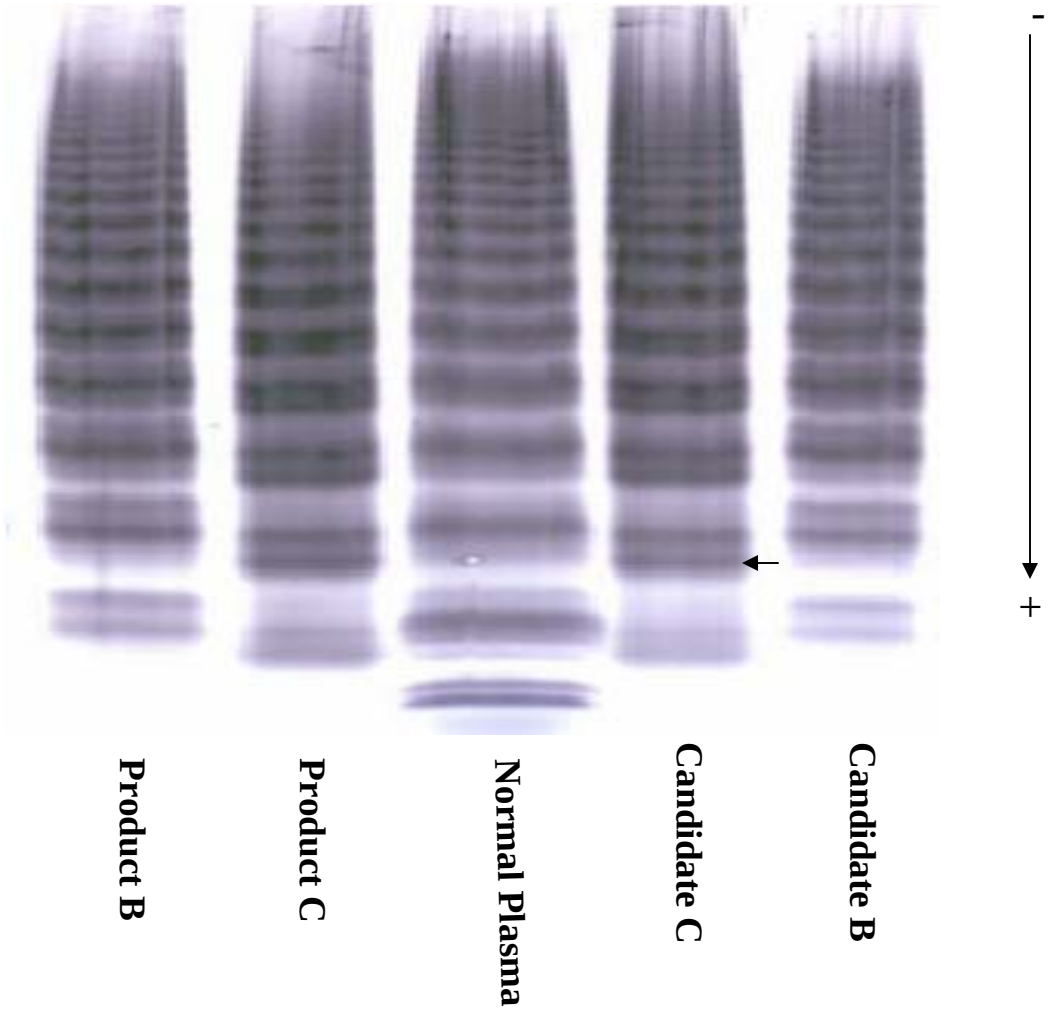
* The assumption was made that the ratio close to one will be the best. The best score is five, and the worst score is one.

Conclusion

- According to the secondary criteria used for the selecting process, the concentrates C-1 and C-3 have the best relevant characteristics for the candidates of the first international standard for the VWF concentrates.

Phase II: Production and Calibration of Standard

- Candidate Materials
  - CANDIDATE (00/514) - coded B in the collaborative study
    - mean fill weight 1.0050 g (range 1.0034 - 1.0076 g)
    - CV of fill 0.08 %
    - mean residual moisture 1.322 %
  - CANDIDATE (00/482) - coded C in the collaborative study
    - mean fill weight 1.0082 g (range 1.0016 - 1.0153 g)
    - CV of fill 0.15 %
    - mean residual moisture 0.397 %

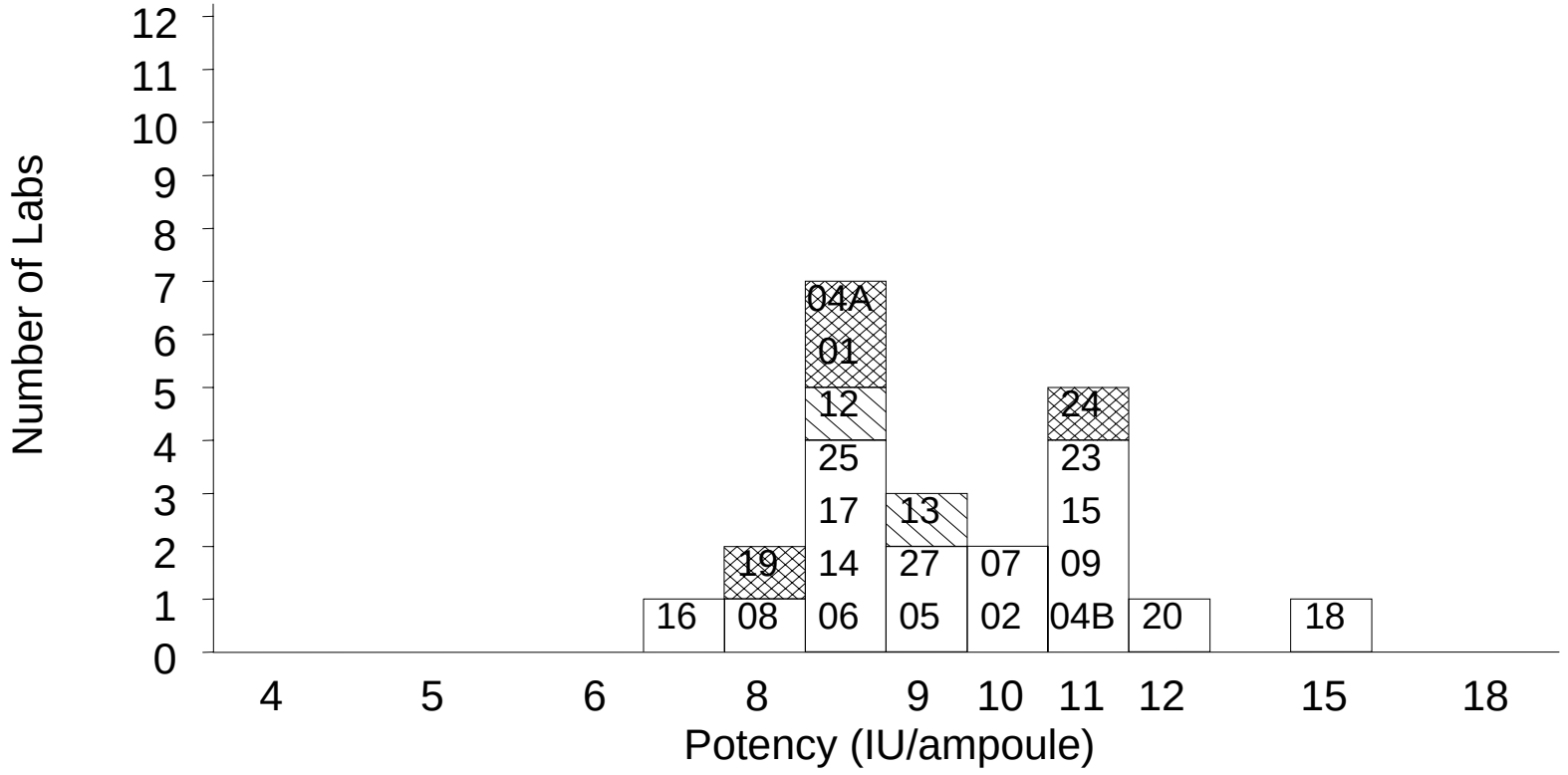


Multimeric pattern obtained using agarose gel electrophoresis (1.5%) of candidates B and C, normal plasma and therapeutic products used to prepare candidates B and C. The arrow points to a band with increased intensity in candidate C indicative of proteolysis.

Phase II: Production and Calibration of Standard (Cont.)

- OBJECTIVES OF THE STUDY
  - Calibration of two candidate VWF concentrates (coded B and C) by assay relative to the 4th IS FVIII/VWF plasma (coded A)
    - VWF:Ag
    - VWF:RCo
    - VWF:CB
  - Calibration of 4th IS FVIII/VWF plasma for VWF:CB
    - by assay relative to local frozen normal plasma pools

VWF: Ristocetin cofactor
Estimates for candidate B relative to the 4th IS plasma (A)



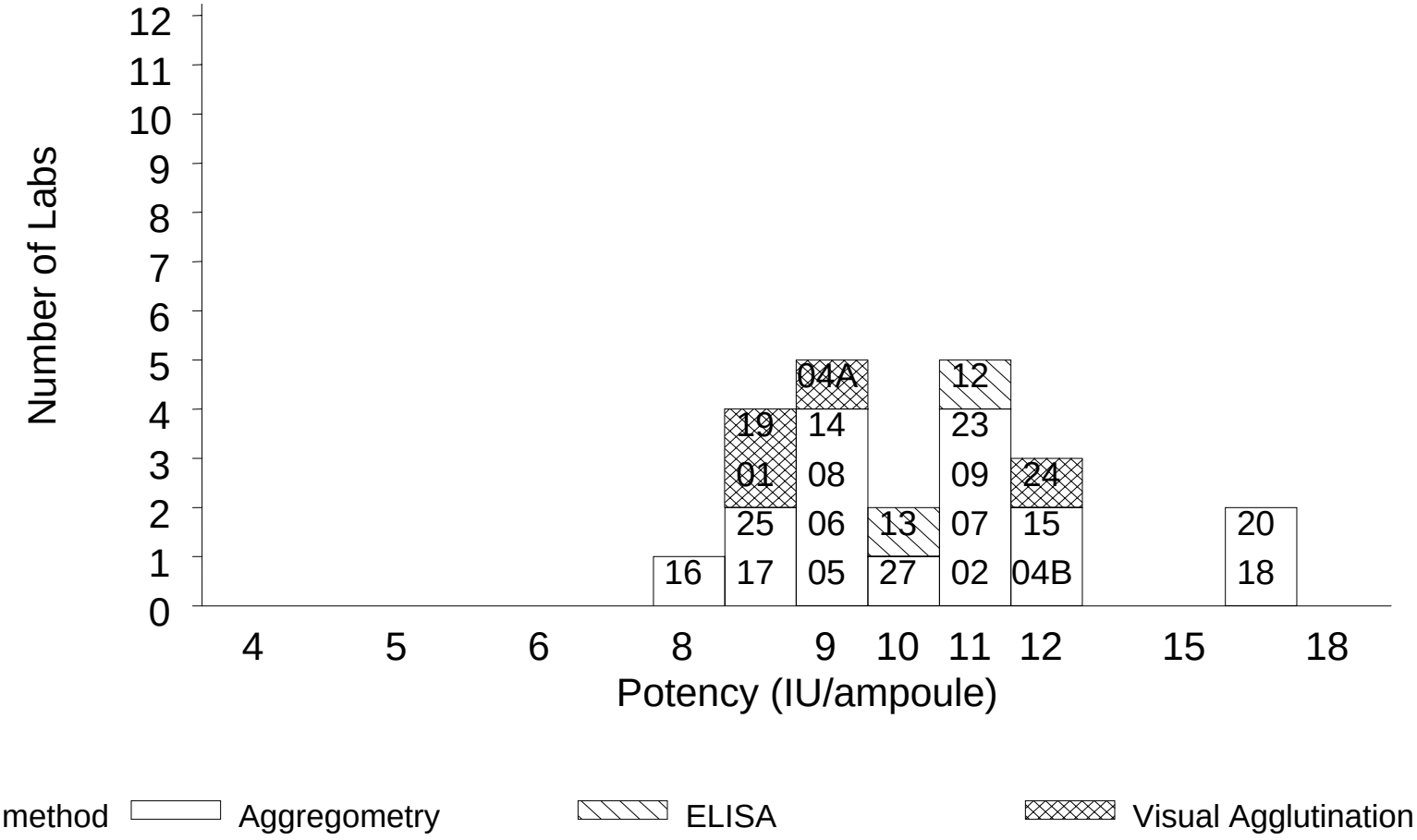
Summary of VWF:RCo Calibration Study
Candidate B vs. 4th IS plasma

Assay method variability	n	Mean estimate (IU/ampoule)	Inter-lab GCV%
Aggregometry	16	9.60	24.2
Visual Agglutination	4	8.51	20.9
ELISA	2	8.70	4.8

- no significant differences between methods

OVERALL MEAN	22	9.31	22.6
exc ELISA	20	9.38	23.7

VWF: Ristocetin cofactor
Estimates for candidate C relative to the 4th IS plasma (A)



Summary of VWF:RCo Calibration Study
Candidate C vs. 4th IS plasma

Assay method variability	n	Mean estimate (IU/ampoule)	Inter-lab GCV%
Aggregometry	16	10.42	25.1
Visual Agglutination	4	9.34	21.1
ELISA	2	10.19	4.2

- no significant differences between methods

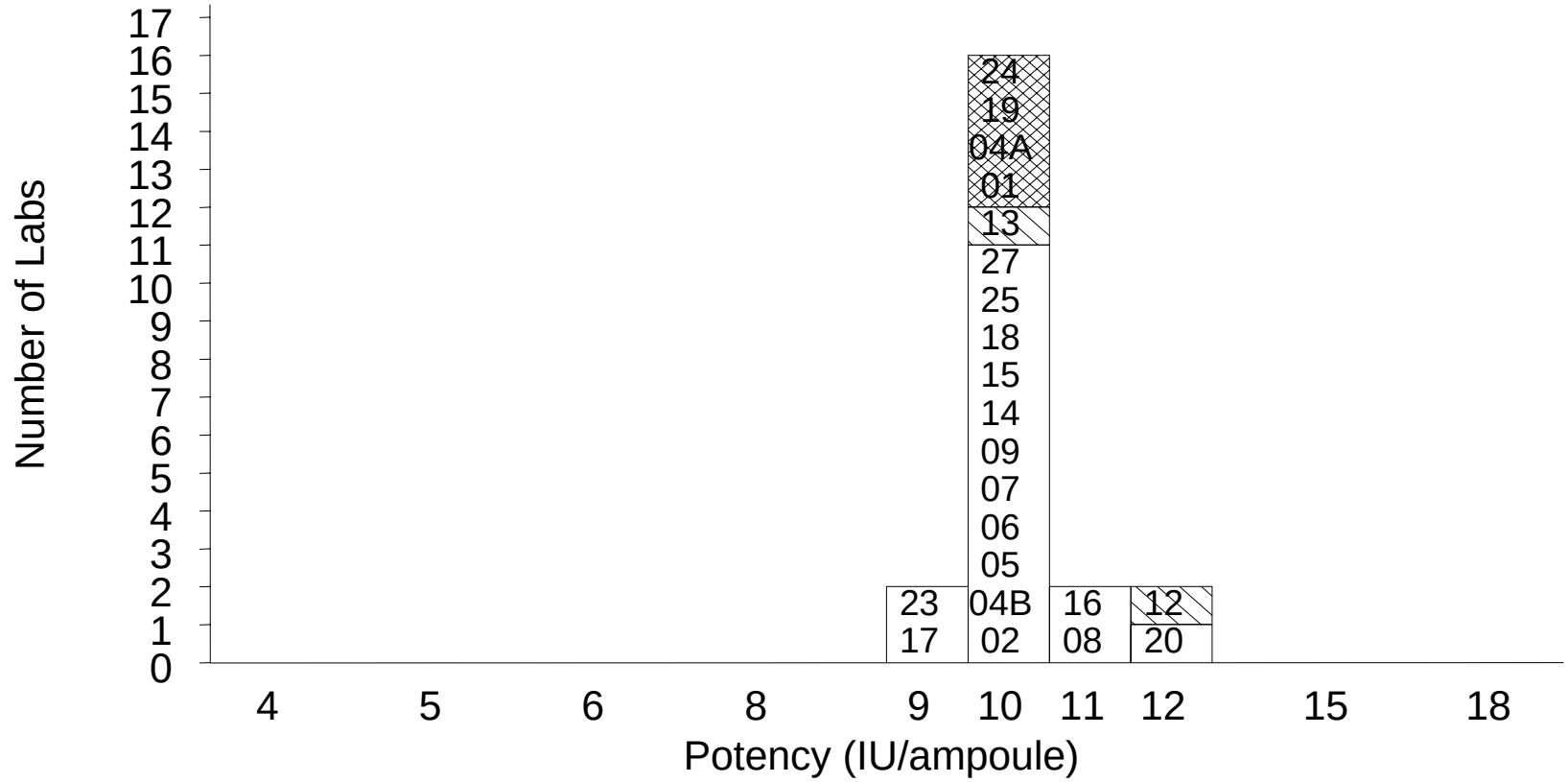
OVERALL MEAN	22	10.19	23.1
exc ELISA	20	10.19	24.4

VWF:RCo – Proposed Potencies

CANDIDATE B **9.4 IU per ampoule** **GCV 23.7%**

CANDIDATE C **10.2 IU per ampoule** **GCV 24.4%**

VWF: Ristocetin cofactor
Estimates for candidate C relative to candidate B



Assay method — Aggregometry — ELISA — Visual Agglutination

Would a Concentrate Standard Reduce Inter-Laboratory Variability of VWF:RCo Estimates?

- Comparison of C vs. 4th IS plasma and C vs B

Assay method	n	Mean estimate (IU/ampoule) and GCV%	
		vs. 4th IS plasma	vs. candidate B
Aggregometry	16	10.42 (25.1%)	10.21 (6.2%)
Visual Agglutination	4	9.34 (21.1%)	10.29 (1.0%)
ELISA	2	10.19 (4.2%)	10.99 (9.2%)
OVERALL MEAN	**22**	**10.19 (23.1%)**	**10.29 (6.0%)**
exc ELISA	**20**	**10.19 (24.4%)**	**10.22 (5.5%)**

The First International Standard for VWF Concentrates

CANDIDATE B

- **Most like plasma VWF**
  - **VWF:RCo/VWF:Ag ratio 0.85**
  - **loss of HMW multimers but no obvious proteolysis**

CANDIDATE C

- **Most like other products included in preliminary studies**
  - **VWF:RCo/VWF:Ag ratio 0.73 vs other products 0.43, 0.74, 0.64**
  - **loss of HMW multimers with some proteolysis**

- WHO Expert Committee on Biological Standardization (ECBS) selected candidate B as the first international standard for VWF concentrates with the following assigned volumes:

VWF:Ag 11.0

VWF:RCo 9.4

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  - *All participating labs.*
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